Asian Journal of Chemistry Vol. 19, No. 5 (2007), 3979-3990

Effect of 15 Days Zinc Loading upon Zinc, Lactic Acid and Creatine Kinase Levels of Wrestlers

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In order to investigate the effect of zinc loading upon the performance of the sportsmen, some wrestlers were subjected to a 15 d zinc loading and completion period training and their effects on lactic acid, zinc and creatine kinase levels were monitored at rest and after heavy exercise. The study covered 20 wrestlers in University wrestling team. The average ages of the wresters was 23.1 ± 2.46 years for group I and 20.8 ± 1.16 years for group II. The average weights and height of the groups I and II are 81.4 ± 18.18 kg and 173.50 ± 9.61 cm and 72.6 ± 5.67 kg and 169.47 ± 8.65 cm, respectively. The $MaxVO_2$ values of group I and group II were 2.82 ± 0.20 L/min and 2.70 ± 0.10 L/min, respectively. Group II (the control group) was given fruit juice during the 15 d experimental period while the Group I (the experimental group) was administered 2 mg/kg day Zn with fruit juice in the same period. The blood samples necessary for the determination of plasma zinc, lactic acid, creatine kinase and hemograms levels were taken from the wrestlers prior to the zinc loading at resting (D1, D2) and after being subjected to a cycling exercise(D2, K2). The second stage was taking the blood samples after 15 d of loading zinc again at resting (D3, K3) and after cycling exercise (D4, K4). The arithmetic means and standard deviations of the data obtained were computed and 't' test was applied to the differences between the dependent and independent groups and the data were evaluated at $p < 0.05$ and < 0.01 significance level. It was determined that the administration of zinc increased the muscle strength of the wrestlers and has a positive effect against exhaustion and on performance by inhibiting the lactic acid release following a 15 d competition period training. The increased levels of zinc were both due to the administration of zinc and heavy training. However the CK levels of the wrestlers were found to be effected from the training but insensitive to zinc loading.

Key Words: Zinc loading, Creatine kinase, Lactic acid, Anaerobic performance, Wrestling.

INTRODUCTION

The human body contains almost all the minerals present in nature. This shows that minerals are of vital importance for a healthy life. The body may not carry its vital functions properly without them. There are

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more than 15 minerals which are essential for human health. Minerals work in cooperation with vitamins and help them to reach the parts where they are needed most. Minerals are necessary to adjust the blood pressure, maintain the rhythm of hearth, muscle functions, maintenance of the liquid balance in the body, reproduction and immune systems. The scientific research showed that the loss or lack of minerals directly effect the human health.

Zinc is one of the most important minerals due to the fact that it takes part in the structure of more than 300 metalloenzymes. Zinc works as a spark which activates most of the functions for a healthy body. It takes role in the formation of RNA, DNA and protein synthesis, self healing and sustaining process of the body, the stability of blood and maintenance of alkaline balance in the body[1-3]. There is *ca.* 2-3 g of zinc in human body. The suggested intake of zinc is 15 mg per day for males. It was reported that the intake of twice or three times of the suggested daily dose of zinc sulfate have positive effect on the body[2,4].

The studies on the animals showed that zinc improves the skeletal system and has a positive effect upon the resistance against fatigue[5]. Krotkiewski *et al.*[6] investigated the effect of administration of zinc upon muscle functions. They gave 135 mg zinc to 16 women for 16 d and compared the experimental group with the control group and suggested that the administration of zinc improved the performance and accelerated the muscle development. Lukaski *et al.*[7] in his study stated that the acute and heavy exercise increases the mineral removal from the body, the Zn levels in plasma remains low for a certain period after the exercise and Zn and Mg support would be necessary to increase the muscle strength.

There is a short term effect upon zinc levels. However prolonged exercises were found to have long term effect on zinc metabolism. The plasma zinc levels of the sportsmen were found to be lower than normal after prolonged exercises. There may be a decrease in muscle zinc concentration as a result of low zinc plasma concentration. The low muscle zinc concentration may be detrimental to endurance capacity since zinc is necessary for the function of so many metabolic enzymes in the body[5].

EXPERIMENTAL

Test subjects

There were 20 wrestlers in Nigde University Wrestling team participated this study carried out to investigate the effect of 15 d competition training and zinc administration upon the zinc, lactic acid and creatine kinase plasma levels of elite wrestlers after heavy training period. Ten of the wrestlers were selected as the experimental (group I) and ten of the wrestlers were selected as the control group (group II). The average age,

weight and height of group I were 23.1 ± 2.46 years, 81.4 ± 18.18 kg and 173.50 ± 9.61 cm, respectively. The corresponding values for group II were 20.8 ± 1.16 years, 72.6 ± 5.67 kg and 169.47 ± 8.65 cm, respectively. The $MaxVO_2$ values of group I and group II were 2.82 ± 0.20 L/min and 2.70 ± 0.10 L/min, respectively. The subjects were adequately informed about the tests and blood analysis they were going to be subjected throughout the experiment.

The following definitions were made for practical purposes:

group I: Experimental group; group II: Control group.

D1: Blood analysis results of the experimental group at rest prior to Zn administration.

D2: Blood analysis results of the experimental group prior to Zn administration after a cycling exercise following training.

D3: Blood analysis results of the experimental group at the end of Zn administration and 15 d competition training period at rest.

D4: Blood analysis results of the experimental group Zn administration and 15 competition period training after a cycling exercise.

K1: Blood analysis results of the control group at rest.

K2: Blood analysis results of the control group after the cycling.

K3: Blood analysis results of the control group at the end of 15 competition training period at rest.

K4: Blood analysis results of the control group at the end of 15 competition training period following a cycling exercise.

The equipment used for the experiment were the following: Monark Bicycle Ergometer for $MaxVO_2$ measurements and exercise protocol. Heart Rate Monitor (Tantuni Brand Telemeter) to measure the heart beat rate of subjects during $MaxVO_2$ measurement. Hand Chronometer (Casio) used for $MaxVO_2$ measurements. Injector with heparin for taking the blood samples. Prime Photometer Spinreact kit for the analysis of the blood samples (in the determination of lactic acid and CK levels). Atomic Flame Absorption Spectrometer for the determination of zinc levels in the blood samples.

Determination of maximum oxygen consumption ($MaxVO_2$): The $MaxVO_2$ values of the subjects were determined by the use of Astrand Bicycle Ergonometer test. The subject turned the pedal of the equipment for 5 min or two successive 2 min unless the heart beat rate differed by only four in two successive readings. The heart beat rate of the subject was monitored by telemeter. The pedal revolving rate was adjusted to 50 rpm. The initial load was adjusted to 150 watt (900 kpm). The heart beat rates of

the subjects were monitored at the end of each min. It was supposed to range between 120 and 170 beat/min. The load was increased by half when the pulse rate was not increased to 120 beat /min in 2 min. On the other hand if the pulse rate increased above 170 beat/min in 3 min the load was decreased by half. The test was continued unless two consecutive pulse rates were approximately the same. The maximum oxygen consumption rate was computed by the use of pulse rates obtained and the load employed.

Exercise protocol: The subjects conditioned themselves with zero load on bicycle ergometer for two minutes and they paddled by the use of 125 % of determined $MaxVO_2$ at a paddling rate of 60 rpm until they got exhausted[9]. This test was applied following the training period before and after the zinc administration and competition period training. Hand Chronometer (Casio) was used in $MaxVO_2$ measurements.

Administration of zinc and normal diet: In the study the group II (the control group) was given pure fruit juice and group I (experimental group) fruit juice containing 2 m/kg Zinc for a period of 15 d. The groups had been given 15 d competition period training. Both groups were thinking that they were given zinc.

Taking blood samples: 10 cc blood samples necessary for the blood test and determination of creatine and zinc levels were syringed out from antecubilatis of subjects from both groups. The samples were taken prior to and after the administration of Zn (D1, K1) at rest and following the cycling exercise(D2, K2) and after the administration of zinc and 15 d competition training period (D3, K3) at rest and following the cycling exercise (D4, K4).

Blood analysis: The blood samples taken from the subjects were centrifuged first and the plasma zinc levels were determined in Biochemistry department of Gazi Medicine School by the use of Atomic Flame Absorption Spectroscopy (Flame AAS; Unicam939-AAS). The plasmas were deprotonized with 25 % (v/v) TCA. A calibration curve was obtained by reading the absorption values of standards prepared with different Zn concentrations (50-200g μgZn/mL) against a blank containing 25 % (v/v) TCA. The deprotonised plasmas were then measured in the same manner. The results were given as mg Zn/dL plasma. Lactic acid and creatine kinase levels were determined in mmol/L with prime photometer by the use of Spinreact kit.

Statistical analysis: The standard means, standard deviations, t-test of the differences of arithmetic means belonging to dependent groups of the data obtained in the study was computed by the use SPSS 10,0 statistical software and they were determined at $p < 0.05$ and < 0.01 significance level.

RESULTS AND DISCUSSION

The comparison of D1-K1 and D2-K2 values revealed that there have been significant differences in the results obtained for group II ($p < 0.01$, $p < 0.05$). The comparison of D3-K3 and D4-K4 values show that there is an insignificant decrease in favour of group II but there is a significant difference in the values of Zn administrated group ($p < 0.01$). When D1-K1 and D3-K3 Zn values are compared one can see that there is a significant difference in the values of group II ($p < 0.01$). The comparison D2-K2 and D4-K4 Zn values indicated significant difference between the Zn values of both groups ($p < 0.05$). There is significant difference in favour of group I as a result of the comparison of D2-K2 values (Table-1).

TABLE-1
COMPARISON OF Zn LEVELS OF THE GROUPS (μg Zn/dL)

Variables	D1-K1		D2-K2		t	Significance level
	X	SD	X	SD		
Group II	86.550	14.31	75.960	8.78	3.433	0.007**
Group I	84.420	7.72	87.330	15.39	-0.632	0.543
T	-0.414	–	2.028	–	–	–
Significance level	0.684	–	0.058*	–	–	–
Variables	D3-K3		D4-K4		t	Significance level
	X	SD	X	SD		
Group II	68.880	14.16	62.280	13.80	2.197	0.056
Group I	78.090	12.88	72.420	13.83	3.261	0.010**
T	1.521	–	0.759	–	–	–
Significance level	0.146	–	0.118	–	–	–
Variables	D1-K1		D3-K3		t	Significance level
	X	SD	X	SD		
Group II	86.550	14.31	68.880	14.16	4.855	0.001**
Group I	84.420	7.72	78.090	12.88	1.976	0.080
T	-0.414	–	0.890	–	–	–
Significance level	0.684	–	0.146	–	–	–
Variables	D2-K2		D4-K4		t	Significance level
	X	SD	X	SD		
Group II	75.960	8.78	62.280	13.80	3.521	0.007**
Group I	87.330	15.39	72.420	13.83	2.864	0.019*
T	2.028	–	1.640	–	–	–
Significance level	0.058*	–	0.118	–	–	–

*$p < 0.05$ ** $p < 0.01$.

D1-K1 and D2-K2 lactic acid values of the groups were compared and it was determined that the increase in D2-K2 values was significant ($p < 0.001$) and however there was no difference between the values of the both groups. The comparison of D4-K4 values of the groups indicated that there was a statistically significant difference between both groups ($p < 0.001$). The D1-K1 and D3-K3 lactic acid values of the groups were compared with each other and it was found that there was not a statistically significant difference between them. The comparison of D2-K2 and D4-K4 lactic acid values of the groups revealed that there was a decrease in the final measurement compared with the initial values and this decrease was significant in D4-K4 values of group II ($p < 0.05$) (Table-2).

TABLE-2
COMPARISON OF LACTIC ACID VALUES OF THE GROUPS mmol/L

Variables	D1-K1		D2-K2		t	Significance level
	X	SD	X	SD		
Group II	2.450	0.35	7.530	2.28	-8.113	0.000**
Group I	2.360	0.28	7.300	2.19	-7.131	0.000**
T	-1.121	–	0.822	–	–	–
Significance level	0.132	–	0.422	–	–	–
Variables	D3-K3		D4-K4		t	Significance level
	X	SD	X	SD		
Group II	2.320	0.28	6.310	2.19	-6.032	0.000**
Group I	2.260	0.60	6.040	1.47	-6.380	0.000**
T	-0.254	–	-0.325	–	–	–
Significance level	0.802	–	0.749	–	–	–
Variables	D1-K1		D3-K3		t	Significance level
	X	SD	X	SD		
Group II	2.450	0.35	2.320	0.28	3.221	0.412
Group I	2.360	0.28	2.260	0.60	0.464	0.654
T	-1.121	–	-0.254	–	–	–
Significance level	0.132	–	0.802	–	–	–
Variables	D2-K2		D4-K4		t	Significance level
	X	SD	X	SD		
Group II	7.530	2.28	6.310	2.19	3.095	0.013*
Group I	7.300	2.19	6.040	1.47	1.603	0.143
T	0.822	–	-0.325	–	–	–
Significance level	0.422	–	0.749	–	–	–

*$p < 0.05$ ** $p < 0.01$.

Data presented in table compares D1-K1 and D2-K2 CK values of the groups. There was a significant difference between D1-K1 and D2-K2 CK values of the both groups ($p < 0.01$). The groups were also compared as regards to their D3- K3 and D4-K4 CK values. It was observed that there was a significant difference between the CK values of both groups before and after the exercise ($p < 0.05$). The comparison of D1-K1 and D3-K3 CK values showed no statistically significant change between the CK values of the groups at rest. The comparison of the groups with each other gave a statistically significant difference ($p < 0.05$). There was not a statistical difference in self values of the groups in the comparison of D2-K2 and D4-K4 CK values. There was also no statistical difference between the D4-K4 values of both groups (Table-3).

TABLE-3
COMPARISON OF CK VALUES OF THE GROUPS (U/L)

Variables	D1-K1		D2-K2		t	Significance level
	X	SD	X	SD		
Group II	227.700	58.02	310.300	96.79	-3.543	0.006**
Group I	268.700	170.63	311.200	182.20	-6.471	0.000**
T	0.719	–	0.014	–	–	–
Significance level	0.481	–	0.989	–	–	–
Variables	D3-K3		D4-K4		t	Significance level
	X	SD	X	SD		
Group II	221.200	63.13	249.900	68.96	-4.02	0.003*
Group I	266.290	210.46	287.550	222.61	-4.05	0.002*
T	0.648	–	-0.511	–	–	–
Significance level	0.525	–	0.616	–	–	–
Variables	D1-K1		D3-K3		t	Significance level
	X	SD	X	SD		
Group II	227.700	58.02	221.200	63.83	0.188	0.855
Group I	268.700	170.63	266.290	210.46	0.055	0.957
T	0.719	–	2.048	–	–	–
Significance level	0.481	–	0.055*	–	–	–
Variables	D2-K2		D4-K4		t	Significance level
	X	SD	X	SD		
Group II	310.300	96.79	249.900	68.96	1.528	0.161
Group I	311.200	182.20	287.550	222.61	0.481	0.642
T	0.014	–	0.511	–	–	–
Significance level	0.989	–	0.010	–	–	–

*$p < 0.05$ ** $p < 0.01$.

The cycling exercise performance times of the groups were compared with each other. The statistical analysis showed that there was a statistically significant difference between D2-K2 and D4-K4 values of the groups and it was seen that there was a significant difference in favor of D4 in D4-K4 values. ($p < 0.05$ and $p < 0.01$) (Table-4).

TABLE-4
COMPARISON OF THE CYCLING EXERCISE TIMES OF THE GROUPS (dk)

Variables	D2-K2		D4-K4		t	Significance level
	X	SD	X	SD		
Group II	3.160	0.94	3.820	1.04	-5.81	0.00**
Group I	2.680	0.35	2.910	0.66	-3.43	0.007**
T	1.570	–	2.330	–	–	–
Significance level	0.134	–	0.003*	–	–	–

*$p < 0.05$ ** $p < 0.01$.

This study was carried out to determine the effect of 15 d competition period training and zinc loading upon the post maximal exercise zinc, lactic acid and creatine kinase levels of the wrestlers. The comparison of D1-K1 and D2-K2, Zn showed that there was a significant decrease in favour of group II in final measurements ($p < 0.01$).

The comparison of D3-K3 and D4-K4 values revealed that there was a statistically decrease in values of groups I in final measurements. Although there was a decrease in the final measurements of group II it was not statistically significant. When we look at the initial measurements of group I and group II we see that the values obtained for group I was higher but it was not statistically significant. The initial measurements of group I was higher and it was attributed to the administration of zinc. The fact that the final measurement is lower than the initial measurements for both groups prove that the training decreases the zinc levels.

Some of studies in literature[5,10,11] state that plasma zinc levels decrease with exercise and it was attributed to the distribution of zinc from the plasma towards the liver and discharge of zinc from the body. In others studies related to discarding of zinc, it was stated that the zinc levels in sweat and urine increased in post training period[12-15]. The fact that there was not a statistically difference in D1-D3 values of group I while there was a statistically significant change in K1-K3 values of group II make us think that the administered Zn increases the potential Zn amount in the organism. In a study carried out by Lukaski *et al.*[7], five healthy males were given Zn containing diet for 30 d, followed by Zn deficient diet for 120 d and finally Zn support for 30 d. The Zn levels were found to show a significant

increase in the days where Zn support was made. The Zn levels were significantly lower in the days when Zn deficient diet was applied[8]. In Ozyürek's study which he carried out on rats the Zn levels of the zinc supported groups were found to be higher than those not given any zinc support[16].

When we compare D1-K1, D3-K3 and D2-K2, D4-K4 zinc value of the two groups there was a statistically significant difference between KI and K3 values of group (II) ($p < 0.01$). The difference was in favour of K3. Although there was not a statistically significant difference between D1 and D3 measurements of group I, D3 values are lower than D1 values. The reason that final zinc values were lower in spite of zinc administration for 15 d competition period training. The fact that the difference between group II measurements were significant while those of group I were not, shows that the decrease in zinc levels of group I was lower than those of group II in spite of the fact that they were subjected to same form of practice. This was attributed to administration of zinc.

The comparison of D2-K2 and D4-K4 values of both groups revealed statistically significant differences between them ($p < 0.01$).

The investigation of the effect of prolonged exercise upon Zn metabolism showed that the Zn levels of the training people were lower that those of control group. The resting Zn levels of the sportsmen and women who carried out prolonged exercise were reported to be lower than those of sedentaries[17]. There are also studies stating that the increased metabolism caused deficiency in zinc which necessitates the support of zinc[18].

When we compare of lactic acid values D1-K1 and D3-K3 measurements indicate that the increase in lactic acid levels after the exercise was statistically significant ($p < 0.001$).Exhaustive exercises result in the use high level anaerobic energy metabolism which causes lactic acid accumulation[9,19-23]. On the other hand there observed no differences between the lactic acid values before the exercise. This shows that there is a balanced distribution in the groups.

The comparison of D2-K2 and D4-K4 lactic acid values of the groups revealed that although there is a decrease in the final measurements compared with the initial measurements there is not a difference with statistical significance. The decrease in D4-K4 lactic acid values is thought to stem from 15 d competition period training.

In a study of Gunduz *et al.*[23] it was found that the lactic acid levels showed a gradual decrease in training for competition as the competition date was approached. It was stated that the biochemical changes in muscle system as a result of endurance exercises results in the increase of the anaerobic capacity and decrease in lactic acid levels[6]. The decrease in the lactic acid levels as a result of training was attributed to the increased rate of lactic acid removal not to the decrease in the rate of lactic acid formation[10,24,25].

There observed no statistically significant change in CK levels at rest after the comparison of D1-K1 and D3-K3 CK values of the individual groups. However the comparison of the groups with each other indicated a difference with statistical significance ($p < 0.05$).

The D3-K3 and D4-K4 CK comparison of the groups revealed that there were statistically significant differences between the pre and post exercise CK levels ($p < 0.05$).

There were significant differences between the groups in both applications. The studies indicate that the exercises damage the muscle fibers and this results the passage of specific muscle enzymes to the circulatory system. Major portion of the total CK amount in the body is present in the skeletal system. The increase in CK blood levels is the indication of muscle damage[26,27]. The studies report that the increase in CK levels was the result of exercise dependent muscle damage. In this study, the Zn support was found to have no effect on increase in CK.

When we compare D2-K2 and D4-K4 CK levels of the groups it is seen that there is increase in K4 values of group II. Although there is an increase in D4 values of group I it is not statistically significant. The increase in D4 CK values of group I was attributed to the fact that some of the wrestlers forming the group had been qualified to the finals 24 h before the measurements.

When there is a muscle damage the plasma the serum activity of a intracellular enzyme CK increases[28,29]. The place where the CK shows the maximum activity is the skeletal muscle. The studies indicate that the CK level reaches to its maximum level 2-4 d after the exercise[30]. The CK level was found to reach its maximum level in 3-4 d after a leg resistance exercise[31]. In Clarkson's study there was eccentric contraction was applied to leg flexors of young and old women and their CK levels were determined. The levels of the elder women were high at the 5th day and the CK levels of the younger women showed a decrease[13]. In a study of Schneider *et al.*[30] the CK levels of marathon runners were found to 21 times higher after the race compared with level measured before it and reached its normal levels 4 d after the race. In another study, the effect of walking up and down hill upon CK levels was investigated with a 13º sloped running band. In an up hill exercise the CK level reached to its maximum value (60-200 IU/L) 24 h after the exercise while in down hill exercises this period was 4-7 d (700-1500 IU/L). The study stated that the eccentric contraction induced more muscle damage than the concentric contraction[32]. The comparison D2-K2 and D4-K4 values or the cycling performance times of the groups indicated a statistically significant difference between the groups. The significance was found to be in the favour of D4 in D4-K4 measurements.

In animal studies it was found that zinc develops the skeletal muscles and increases the resistance against fatigue[5]. Krotkiewski *et al.*[6] investigated the effect of zinc upon muscle functions. 16 Women were loaded 135 mg zinc per day for 15 d. The results indicated that the administration of zinc resulted in the increase in performance and improved muscle development and muscle repair.

Lukaski *et al.*[7] stated that acute and heavy exercise increases the mineral removal from the body with sweat and urine but returns to normal within few days. He also emphasized that zinc and magnesium support increases the muscle strength and strengthens the muscle metabolism. The plasma zinc concentration continues to decrease for a certain time after an exercise. It is stated that daily and continuous exercises may damage zinc metabolism and the loss of zinc may cause muscle exhaustion and loss of strength[33,34]. Tamer[16] stated that the exercises carried out with aerobic capacity were directly related with their density, time and frequency. He claims that the physical condition can be improved by the exercises carried out with 50-85 % of MaxVO_2 five times a week 15-60 min a day.

As apparent from the literature, zinc increases the muscle strength has a positive effect against exhaustion, accelerates the curing period of the contracted muscles and improves muscle development. The competition period training is assumed to result in the biochemical changes in the muscles, increase the aerobic capacity and improve the performance.

In conclusion, this study is carried out to evaluate the effect of zinc administration and competition period upon post heavy exercise lactic acid formation levels of elite wrestlers it was found that zinc loading increased the muscle strength and has a positive effect against exhaustion, increased the relaxation rate of the contracted muscles and improved their development and decreased the lactic acid release in muscles together with heavy competition period training which resulted biochemical changes in muscle structure and increased the performance. The increase in Zn levels was attributed to zinc support in addition to heavy training. The CK values were found to be effected by training but insensitive to zinc loading.

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(*Received*: 14 October 2006; *Accepted*: 9 March 2007) AJC-5507